Brief Communication

Eimeria rangiferis (Protozoa: Eimeriidae) reported from caribou in Ameralik, West Greenland

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Abstract: In recent decades the native Barren-ground caribou (*Rangifer tarandus groenlandicus*) south of the Godthaabs fjord (Nuup Kangerlua fjord) in West Greenland have mixed with semi-domesticated Norwegian reindeer (*R. t. tarandus*) imported in 1952 from Finnmark Norway and released onto the range of the Ameralik caribou population. Fecal samples from three calves of the Ameralik caribou population were examined for the presence of nematode eggs and eimerid oocysts. Two distinct nematode egg types were observed: the first, *Nematodirella longissimespiculata*, was found in all calves, while the second, a strongylid nematode, was detected in one calf. The coccidian eimerid *Eimeria rangiferis* was identified in all calves. This host-specific eimerid is found in Icelandic feral reindeer, which were also imported from Finnmark Norway. We suggest that Finnmark reindeer were the source of *Eimeria rangiferis* observed in Ameralik caribou today. There are three possible origins for the presence of *N. longissimespiculata* in Ameralik, 1) arrival with colonizing caribou from North America within the past 4000 years, 2) the 1952 introduction of semi-domesticated Norwegian reindeer, or 3) the current immigration of muskoxen.

Key words: *Eimeria rangiferis;* fecal analysis; *Nematodirella longissimespiculata;* Norway; *Rangifer tarandus groenlandicus; R. t. tarandus;* West Greenland.

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Introduction

Until 1952 indigenous wild caribou (*Rangifer tarandus groenlandicus*) were the only large herbivore present in West Greenland (ca. 61°-69°N). That year 263 semi-domesticated reindeer (*R. t. tarandus*) from Finnmark in northern Norway were imported to West Greenland and released onto the range of the Ameralik caribou population whose abundance was low (Cuyler, 1999). The Ameralik range includes areas both east and south of the Godthaabs fjord (Nuup Kangerlua fjord) and contains Greenland's capital city, Nuuk. Although initially, hired Norwegian Sami herders strictly contained reindeer distribution to the Itvinera-Kapisillit husbandry area, by 1978 locals had that responsibility. Herd control was lacking, and with each passing year the reindeer expanded further beyond the husbandry district. By the end of the century reindeer occupied the entire Ameralik caribou

range and interbreeding was common (Jepsen *et al.,* 2002, Cuyler *et al.,* 2003). At present the Ameralik population may exhibit morphological characteristics of both subspecies (Cuyler *et al.,* 2007). Recent surveys for abundance of the Ameralik population provided estimates of ca. 10,000 and 12,000 animals for 2006 and 2012 respectively (Cuyler *et al.,* 2007, unpublished).

Recently, Steele *et al.,* (2013a; 2013b) examined intestinal parasites in the adjacent Akia-Maniitsoq population (ca. 64°- 66°N), which is north of Nuuk and separated from the Ameralik caribou by the ≥ 5 km wide Godthaabs fjord. One reindeer-specific helminth, the abomasal nematode *Ostertagia gruehneri*, was identified (Steele *et al.,* 2013b). Fecal samples revealed strongyle-type eggs (presumably *O. gruehneri*), eggs of a Nematodirinae nematode, eggs of an Anoplocephalidae cestode (probably *Moniezia* sp.) and two varieties of *Eimeria* oocysts (Steele *et al.,* 2013a). Similar parasite fauna may be expected in the Ameralik caribou, since a 1995 genetics study revealed that Norwegian semi-domestic reindeer had crossed the Godthaabs fjord and mixed with the Akia-Maniitsoq caribou (Jepsen *et al.,* 2002). Parasitological studies on the indigenous Kangerlussuaq-Sisimiut caribou population (ca. 66°- 68°N), which is yet further north and without contact to other populations in West Greenland (Jepsen *et al.,* 2002), revealed three nematode species (*Nematodirella longissimespiculata, Marshallhagia marshalli, Teladorsagia boreoarcticus)*, perhaps an Anoplocephalid cestode and two morphologically different coccidians (Eimeria spp.) (Clausen *et al.,* 1980; Korsholm & Olesen, 1993; Steele *et al.,* 2013a; 2013b).

Similar to West Greenland, Iceland also received reindeer from Finnmark Norway by the end of the 18th century. Today, six reindeer-specific protozoan parasites (*Eimeria spp*., *Sarcocystis* spp.) are known in Icelandic reindeer (Gudmundsdottir, 2006; Gudmundsdottir & Skírnisson, 2005; 2006; Dahlgren *et al.,* 2007). This confirms a successful long-term transmission of these host-specific parasites after translocation of their hosts.

Our study is the first time intestinal parasites from the Ameralik caribou are examined. We focused on intestinal parasites and searched for nematode eggs and eimerid oocysts. We hypothesize that the 1952 arrival of reindeer from Finnmark to the Ameralik caribou range introduced reindeer-specific parasite fauna, which may be detected today, specifically *Eimeria* spp. We compare our results to those from the nearby Akia-Maniitsoq and distant Kangerlussuaq-Sisimiut populations.

Materials and methods

Since eimerid parasite infections are typically more abundant and easier to detect in young animals (Skírnisson, 2007), fecal samples were obtained from three 4-5 month-old Ameralik calves in the period 6-15 October 2013. The calves (two females, one male) were shot in the Austmannadalen valley (64° 13' 18" N; 50° 03' 18" W) of the inner Ameralik fjord, and ca. 90 km south-east of the Akia-Maniitsoq study area in Steele *et al.* (2013a, b).

Twenty grams feces from the rectum were submerged in 40 ml 3% $K_2Cr_2O_7$ using an 80 ml tight screw cap plastic tube. As eimerid identification and description requires sporulated oocysts, the samples remained at room temperature for three to four weeks until arrival at Keldur Iceland, 5 November 2013, following which they were refrigerated at 4°C until analysis.

To obtain quantitative estimates of the number of nematode eggs and total coccidian (*Eimeria* spp.) oocyst excretion we employed the McMaster method (Anonymous 1986). Three grams of feces were suspended in 87 ml of water. After centrifugation, the supernatant was decanted and the tube refilled with Parasitosol (density 1.27g/ml, Meku®, DK 7171, Denmark) to the same level. Four McMaster

chambers (in two slides) were filled and 0.6 ml examined under a microscope at 125x magnification. Thus, the minimum detectable helminth egg and oocyst number per gram faeces (i.e., epg and opg respectively) were 50.

Owing to top glass thickness of the McMaster chamber, this method permits a maximum 100 - 125x magnification under a microscope. Detailed morphological examination of oocysts and eggs require higher magnification, e.g., 400x, 1,000x, or 1,250x. Therefore, we developed our own "chamber method" that not only enables high magnification examinations, but also facilitates egg and oocyst accumulation immediately underneath a cover glass. Approximately one gram feces was thoroughly mixed with 12ml Parasitosol floatation medium in a 15ml test tube, and subsequently centrifuged at 500x *g* for 10 minutes. This compelled oocysts and eggs to accumulate at the sample surface. Using a plastic pipette (large 4mm aperture) single drops were removed from that surface and transferred, one by one, into a ca. 0.3 ml circular chamber (o-ring gasket, diameter 20mm, sealed on a microscope slide). Once full, the chamber was covered by a 24 x 24mm cover-glass and morphological examinations of eggs and oocysts begun at the desired magnification.

Photographs were made with a Nikon DS-Vi1 digital camera mounted on a Leica DMLB microscope equipped with 12.5x oculars and Nomarski (DIC) contrast. A calibrated eyepiece graticule was used to evaluate oocyst and egg size (length x width in µm). Length measurements and morphological examination were used to distinguish nematode egg type. Large (> 200µm) eggs are of the subfamily Nematodirinae (Fruetel & Lankester, 1989), while medium-sized (< 100µm) are a strongyle nematode (Rommel *et al.,* 2000; Thienpont *et al.,* 1979). Nematodirinae eggs were further identified using the key of Fruetel & Lankester (1989). Identification of eimerids detected in our study followed Gudmundsdottir & Skírnisson (2005; 2006).

Results

Two distinct nematode egg types as well as oocysts of the coccidian *Eimeria rangiferis* were detected in the fecal samples.

Nematodes

Eggs of *Nematodirella longissimespiculata* were observed in all calves. Abundance was below the McMaster method detection level (50 epg). The "chamber method", however, confirmed their presence (Figure 1), with average egg size, 246x120 µm (range 210-280 x 115-125 µm, n= 9). Median egg length was 248 µm.

A second nematode egg type belonging to the group "other strongyles" (Figure 2), was observed in one calf only. Abundance was again below the McMaster method detection level (50 epg), while the "chamber method" confirmed the presence of two eggs, both measuring 97 x 52 µm.

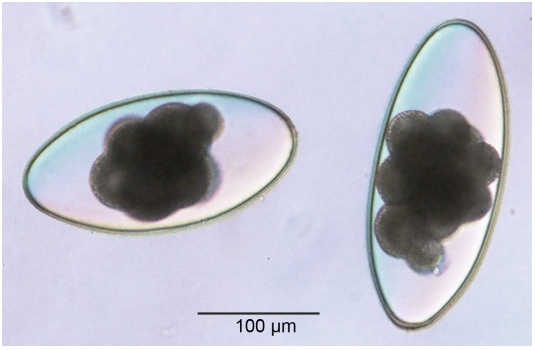

Figure 1. Nomarski interference contrast photomicrograph of two *Nematodirella longissimespiculata* eggs from feces of an Ameralik caribou calf (mixed native caribou and semi-domestic Norwegian reindeer) of West Greenland. The ellipse eggs have a thin smooth surfaced chitinous shell, rather sharp poles and similar side-walls. Eight dark-staining blastomeres are separated from the yolk membrane by a wide fluid-filled cavity. Bar = 100 µm.

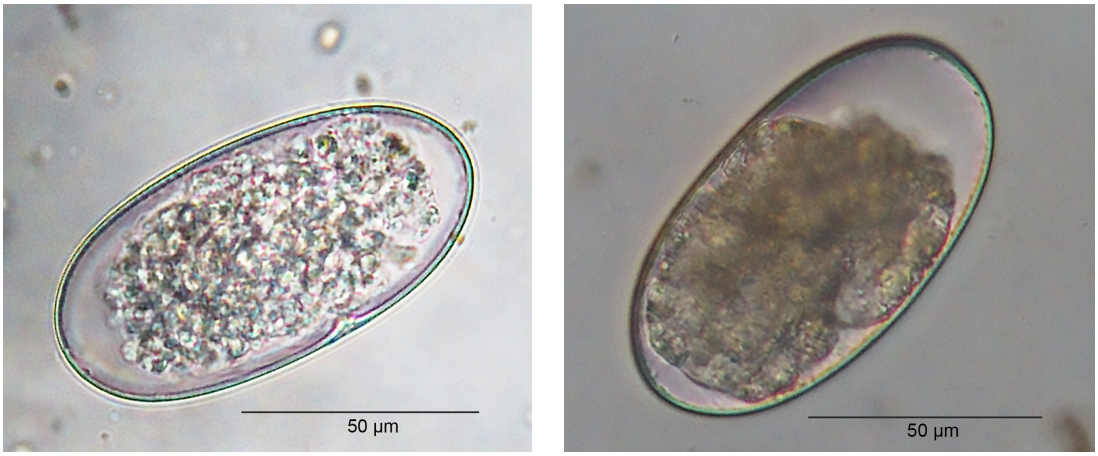

Figure 2. Nomarski interference contrast photomicrographs of two strongyle type nematode eggs from feces of Ameralik caribou calves (mixed native caribou and semi-domestic Norwegian reindeer) of West Greenland. The symmetrical ellipse eggs have somewhat barrel-shaped side walls between two rounded poles. The thin chitinous shell is smooth surfaced. A large number of hardly distinguishable blastomeres occupy the entire decomposing eggs. Bar = 50 µm.

Coccidia

Eimeria rangiferis oocysts (Figure 3) were found in all calves. Abundances were 50, 50 and 650 opg, for the male and two females respectively. Average size (length x width) of the oocysts was 34.5 x 29.0 µm (range 29-40 x 26-34 µm, n=12). For unknown reason(s) only 25% of the oocysts sporulated successfully.

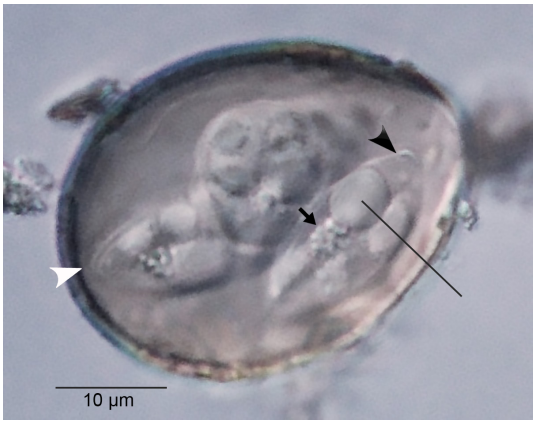

Figure 3. Nomarski interference contrast photomicrograph of sporulated *Eimeria rangiferis* oocyst from feces of an Ameralik caribou calf (mixed native caribou and semi-domestic Norwegian reindeer) of West Greenland. Microphyle on the top of the oocyst is not in focus. Note the sporocyst residuum (**arrow**), 1 refractile body (**line**) in each sporozoite, and the Stieda body (**black arrowhead**). The spindle-shaped sporocyst has a slightly pointed end opposite the prominent Stieda body (**white arrowhead**). Bar = 10 µm.

Discussion

Nematodes

Our study documents for the first time the presence of *Nematodirella longissimespiculata* in the Ameralik caribou population, which is a genetic mix of native caribou and introduced semi-domesticated reindeer. Previously, eggs of an unknown Nematodirinae nematode were reported from caribou in the nearby Akia-Maniitsoq and more distant Kangerlussuaq-Sisimiut caribou populations (Steele *et al.,* 2013a). It remains open if this is the same species. In key to ova of the gastrointestinal helminths of caribou in North America Fruetel & Lankester (1989) report that Nematodirinae eggs with mean length > 240 µm belong to genus *Nematodirella* and mean egg length

differs among regions; eggs < 230 µm in length belong to genus *Nematodirus*. In North America genus *Nematodirus* is represented by three well-known species in caribou; *N. odocoilei* (maximum egg length 180 µm), *N. tarandi* and *N. helvetianus*, (maximum egg length of both species 228 µm). In the present study nematodirinae egg lengths ranged from 210 to 280 µm with average length of 246 µm. However, two eggs, measuring only 210 and 220 µm (one egg each from two calves) indicate that also a representative of genus *Nematodirus* might be present in the Ameralik caribou population. Removing the two shortest eggs, results in a mean and median *Nematodirella* sp. egg length of 255 µm (n=7) for Ameralik caribou in West Greenland. Given our limited sample size, caution is appropriate regarding species identification. Also, there may be parasites present in the Ameralik caribou, e.g., *Marshallagia marshalli*, that went undetected. Further investigation is warranted to definitively ascertain the presence of *Nematodirella longissimespiculata* in Ameralik caribou.

Given that *N. longissimespiculata* occurs in *Rangifer* spp. across North America (Hoberg *et al.,* 2001), this parasite could have arrived to West Greenland with the native caribou population that colonized Greenland thousands of years ago. It has been suggested, however, that the *N. longissimespiculata* reported in the Kangerlussuaq-Sisimiut caribou population (Clausen *et al.,* 1980, reported as *N. longispiculata*; Korsholm & Olesen, 1993) were acquired from the sympatric muskoxen, which were imported from northeast Greenland in 1962 (Boertmann *et al.,* 1992). If the latter is true, then Akia-Maniitsoq and Ameralik caribou may also have recently acquired the *N. longissimespiculata* parasite from those muskoxen. Since the turn of the century, observations confirmed that muskoxen from Kangerlussuaq-Sisimiut were crossing the Sukkertoppen Ice Cap and entering the Akia-Maniitsoq caribou range to the south. Subsequent observations have placed muskoxen ever further south, and since 2005 muskoxen have been observed on the northeastern portion of Ameralik caribou range, several animals were within 10-25 km of our study area. Transmission of *N. longissimespiculata* from muskoxen to Ameralik caribou and our sampled calves has been possible. Finally, *N. longissimespiculata* has been documented in Norwegian reindeer (Josefsen *et al.,* 2014) and could have arrived to Greenland with their 1952 release at Itivnera in the north Ameralik area.

Steele *et al.,* (2013b) reported the presence of the strongyle *Ostertagia gruehneri* in Akia-Maniitsoq caribou, however, it has not been reported in the adjacent Kangerlussuaq-Sisimiut caribou population (Clausen *et al.,* 1980, Korsholm & Olesen, 1993; Steele *et al.,* 2013b). Morphology of our study's strongyle-type eggs (Figure 2) from Ameralik caribou coincides with descriptions of *Ostertagia* eggs (Thienpont *et al.,* 1979; Fruetel & Lankester, 1989). Several strongyle nematodes, however, exhibit similar egg morphology making conclusive identification to genus or species impossible. To explain *Ostertagia gruehneri* presence in Akia-Maniitsoq and absence in Kangerlussuaq-Sisimiut, Steele *et al.* (2013b) suggested *O. gruehneri* was introduced to the Akia-Maniitsoq caribou with the import of semi-domestic reindeer from Finnmark Norway, which are known to carry this parasite (Josefsen *et al.,* 2014). In contrast, today Icelandic reindeer lack *O. gruehneri*. This host-specific nematode may have disappeared from Iceland during the reindeer abundance bottleneck of the early 20th century.

Two further nematodes recently reported in Kangerlussuaq-Sisimiut, but not Akia-Maniitsoq, include *Marshallhagia marshalli* and *Teladorsagia boreoarcticus* (Steele *et al.,* 2013b). Earlier, Korsholm & Olesen (1993) reported *M. marshalli* and *Teladorsagia circumcincta*, however, the putative identity of the latter has

recently been questioned (Hoberg *et al.,* 1999). Steele *et al.* (2013a) suggested *M. marshalli* in Kangerlussuaq-Sisimiut caribou was the result of spill-over from the introduced muskoxen. Given that muskoxen currently inhabit the caribou range of both Akia-Maniitsoq and Ameralik, future research may report *M. marshalli* in these areas.

Coccidia

Eimeria rangiferis was found in all Ameralik caribou calves sampled. Previous studies have reported *Eimeria* spp. oocysts in the Akia-Maniitsoq (Steele *et al.,* 2013b) and Kangerlussuaq-Sisimiut caribou populations (Clausen *et al.,* 1980; Steele *et al.,* 2013b) with Korsholm & Olesen (1993) detecting two different *Eimeria* oocysts in the latter. Unfortunately, no descriptions of these eimerids are available.

In Iceland three reindeer-specific protozoan parasites *Eimeria hreindyria, E. mayeri* and *E. rangiferis* were introduced with the arrival of semi-domestic reindeer from Finnmark Norway (Gudmundsdottir & Skírnisson 2005; 2006). Since the late 18th century saw the last translocation of reindeer to Iceland, these coccidians have survived in the Icelandic reindeer population for over 200 years despite a near extinction of their hosts in the early 20th century. If this situation repeated itself when Finnmark reindeer were imported into West Greenland a century and a half later, then up to three eimerids originating in the Palaearctic could have been introduced to West Greenland.

Future research may enlighten what eimerid species occur in Greenland caribou. We expect that coccidian fauna in West Greenland also includes North American eimerid species that arrived when North American barren-ground caribou colonized the west coast of Greenland 4000-7000 years ago (Roed, 2005; Steele et al., 2013b). Although *Eimeria spp.* occurs in Canadian caribou (Frechette 1979; Susan Kutz & Karl Skírnisson, unpublished), currently no description exists on *Eimeria spp.* in caribou from the Nearctic.

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